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(54) Title: PEPTIDES FROM THE TT VIRUS SEQUENCE AND MONOSPECIFIC ANTIBODIES BINDING TO THE TT VIRUS

(57) Abstract

The peptide having the amino acid sequence SEQ ID NO:1, and optionally this peptide in mixture with one or more peptides SEQ ID NO:3 – 11, is described. All these peptides correspond to regions of the genomic TT virus sequence. Further, monospecific antibodies binding to the TT virus are disclosed. The peptides may be coupled to a carrier and/or label, or immobilized on a solid phase. The peptide or peptide mixture, or the monospecific antibody may be used in a medicament or in diagnostic kits. The peptide or peptide mixture may also be used for immunization of a non-human mammal to produce monospecific antibodies directed against TT virus.

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Peptides from the TT virus sequence and monospecific antibodies binding to the TT virus.

The present invention relates to peptides derived from the genomic TT virus sequence and monospecific antibodies binding to the TT virus. Further, the invention relates to the peptides and antibodies of the invention for respective use in medicaments. Diagnostic kits comprising the peptides of the invention as diagnostic antigens, and diagnostic kits comprising the antibodies of the invention as diagnostic antigens are also comprised by the invention. The peptides of the invention may be used for immunization of a non-human mammal to produce monospecific antibodies directed against TT virus.

Background

In 1997 a novel human infectious agent was identified from the serum of a Japanese patient with post transfusion non A-G hepatitis and named TT virus (TTV) [1]. TTV DNA was detected in 47% of patients with fulminate non-A-G hepatitis and 46% of patients with chronic liver disease of unknown etiology [2] suggesting that TTV may be the cause of some idiopathic liver disease. TTV is global [3]] and seems to be more common in populations with increased risk for infection with blood borne viruses [2] e.g. hemophiliacs and drug addicts. However, non-parenteral transmission seems also to be possible [2].

TTV is a non-enveloped, single stranded DNA virus with a genome of at least 3,7 kb [4]. It has a range of sequence divergence, allowing classification into different genotypes and subtypes [4]. A relationship with the family *Parvoviridae* has been discussed [4]. Subsequent analyses revealed evidence of hepatotropism of TTV [2] and in some patients with non A-G post transfusion hepatitis and TTV viremia TTV DNA titres correlated with aminotransferase levels [1].

However, an evidence for an association between TTV infection and severe liver disease could not be strengthened [3, 5, 6]. The epidemiological, immunological, and clinical significances of TTV infections are still uncertain. Moreover, no serological tests for TTV infection are available yet and at the moment PCR is the only available diagnostic tool.

It would be desirable to be able to diagnose TTV infection in man, and to develop medicaments based on peptides for immunization and/or antibodies against TTV.

Description of the invention

The present invention is based on synthetic peptides that correspond to different regions of the genomic sequence from the recently described TT virus (TTV; [1]). A total of 80 overlapping peptides corresponding to the two open reading frames (ORFs; Genebank

accession no AB008394) 1 and 2 were synthesized. These were analyzed with eight human serum samples with TTV infection and eight human samples without TTV infection. Reactive human serum samples all reacted with a peptide with the sequence SEQ ID NO:1 : TATTTTYAYPGTNRPPV. The reactivities could be fine mapped to the sequence SEQ ID NO:2 : YAYPGTNRPPV where the residues PV were found to be those most essential for the binding of human antibodies.

Thus, the present invention is directed to a peptide having the amino acid sequence SEQ ID NO: 1

TATTTTYAYPGTNRPPV

wherein one to all six of the N-terminal amino acids TATTTT may be omitted.

In an embodiment of the invention the peptide has the amino acid sequence SEQ ID NO:2

YAYPGTNRPPV.

The invention is also directed to a peptide mixture comprising the peptide SEQ ID NO: 1

TATTTTYAYPGTNRPPV

wherein one to all six of the N-terminal amino acids TATTTT may be omitted, and at least one other of the peptides listed in Table 4 having the amino acid sequences SEQ ID NO:3 - 11.

The peptide and/or at least one of the peptides in the peptide mixture of the invention may be coupled to a carrier and/or label. Examples of carriers are plastic surfaces, such as microplates, beads etc.; organic molecules such as biotin; proteins, such as bovine serum albumin; peptide linkers, or polypeptides. Examples of labels that can be used, primarily for diagnostic purposes, are radioactive isotopes, enzymes, fluorescent markers, etc.

Further, the peptide and/or at least one of the peptides in the peptide mixture of the invention may be immobilized on a solid phase, such as a glass or plastic surfaces, primarily for diagnostic purposes or purification of antibodies.

The present invention is also directed to the peptide or peptide mixture of the invention for use in a medicament, optionally coupled to or in combination with other biologically active or inactive ingredients, such as a vaccine for prevention of TT virus infection.

Further, the invention is directed to monospecific antibodies binding to the TT virus.

In an embodiment of the invention, the monospecific antibody binds to an amino acid sequence selected from the group consisting of the amino acid sequences SEQ ID NO:1 - 11.

The invention is additionally directed to a monospecific antibody according to the invention for use in a medicament, optionally coupled to or in combination with other biologically active or inactive ingredients, such as a medicament for administration to a patient already infected with TTV.

The present invention is also directed to a diagnostic kit comprising a peptide or peptide mixture according to the invention as diagnostic antigen(s). The kit may be used in an immunological assay, such as EIA, RIA etc, to detect the presence of antibodies against TTV in a biological fluid, such as blood or plasma.

The invention is further directed to a diagnostic kit comprising one or more monospecific antibody according to the invention as diagnostic antibodies. The kit may be used in an immunological assay, such as EIA, RIA etc, to detect the presence of antibodies against TTV in a biological fluid, such as blood or plasma.

The diagnostic kits will normally comprise additional ingredients for performing an immunological assay. These additional ingredients will depend on the actual assay to be used and will often comprise positive and negative standard serum samples and written instructions for use.

The present invention is additionally directed to the use of a peptide according to the invention for immunization of a non-human mammal to produce monospecific antibodies directed against TT virus.

The present invention will now be further illustrated by reference to the following description of experiments and specific embodiments of the invention, which are not to be considered as limitations to the scope of the invention defined in the claims.

Description of experiments

Serum samples:

Coded serum samples were obtained from a serum bank containing healthy blood donors, children with or without liver disease, mothers with IVDU (intravenous drug use) and their children.

PCR amplification for the detection of TTV DNA in serum:

Total DNA was isolated from 50 µl patient serum by phenol/chloroform purification. The DNA of all patients was analyzed with two different primer settings by (semi) nested PCR. Five µl patient DNA were added to a 45 µl reaction mix containing 1 U

taq polymerase (Perkin-Elmer Applied Biosystems, Norwalk, CO), 10x PCR buffer, 200 µmol $MgCl_2$, dNTPs (125 µmol/nucleotide) and 20 pmol of each primer. The first round primers were 5TTVout5 (5'-ACA GAC AGA GGA GAA GGC AAC ATG- 3') and either 3TTVout (5'- CTG GCA TTT TAC CAT TTC CAA AGT T- 3') or 3TTXout (5'-TAC CAY TTA GCT CTC ATT CTW AT- 3') as downstream primers. The DNA was amplified as follows: 95°C for 4.5 minutes and then 33 cycles of 95°C for 30 sec, 50°C for 30 sec and 72°C for 1 min, and at the end 72°C for 4 min. A second round PCR was performed using 5 µl of the first-round PCR product under identical conditions. The second round inner primers were either 5TTVin (5'-GGC AAC ATG YTR TGG ATA GAC TGG - 3') or 5TTVXin (5'-ACA GGA GAC HMA AAC ATA SA- 3') as upstream primers and 3TTVout. The correct size of about 275 respectively 140 bp was determined by agarose gel electrophoresis (3%). Samples which were either positive with both primer sets or reproducibly positive with one primer set were considered as TTV positive. Primer sequences were based on Genebank accession no AB008394).

Peptide synthesis

Overlapping peptides (18 aa long with a 8 aa overlap) corresponding to the ORF1 and ORF2 of TTV (Table 1; Genebank accession no AB008394) were produced by a multiple peptide synthesizer using standard Fmoc chemistry [7] (Syro, Syntex, Germany).

Detection of human antibodies in serum

The EIAs mainly followed previous protocols [8]. Microplates (Nunc, Denmark) where coated for 48 hours with synthetic peptides at a concentration of 10 µg/ml in 0.05M sodium carbonate buffer pH 9.6. After blocking for 2 hours at room temperature with phosphate buffered saline containing 1% bovine serum albumin, 2% goat serum and 0.05% Tween 20 (dilution buffer) the plates were incubated with human sera diluted 1:100 in dilution buffer. Bound human IgG was indicated by incubation with anti-human IgG antibodies conjugated to alkaline phosphatase (Sigma Chemicals, St. Louis, MO). The plates were developed by the addition of dinitro-phenylene-diamine (Sigma) and the optical densities were determined at 405nm.

Immunization and induction of TTV-specific antibodies

Groups of Balb/c were immunized intra peritoneally with 100 µg of the TTV peptide 35 (SEQ ID NO:1) emulsified 1:1 in complete Freund's adjuvant . A booster dose of 100 µg in incomplete Freund's adjuvant was given four weeks later. Venous blood samples were obtained once a week for six weeks and were tested for reactivity for the TTV peptide 35 (SEQ ID NO:1).

Results

Human reactivities to the 97 peptides covering ORF1 and ORF2 have been given in Tables 2 and 3. Reactive peptides within ORF1 were found to be peptides 10 (SEQ ID NO:3), 18 (SEQ ID NO:4), 29 (SEQ ID NO:5), 35 (SEQ ID NO:1), 42 (SEQ ID NO:6), 44 (SEQ ID NO:7), 50 (SEQ ID NO:8), 51 (SEQ ID NO:9), and 69 (SEQ ID NO:10) (Table 2). Two of the tested human sera were reactive with peptide 19 (SEQ ID NO:11) from ORF2 (Table 3). All reactive peptides have been listed in Table 4. The most often detected peptide was the peptide 35 with the sequence TATTTTYAYPGTNRPPV (SEQ ID NO:1). The reactivity to peptide 35 was dependent on the dilution of the serum samples (Table 5). The reactivity of the human serum samples to the peptide on the microplate could be inhibited by the addition of the same peptide in solution, but not by an irrelevant peptide (data not shown). This shows that the reactivity is specific for the peptide 35 with the sequence TATTTTYAYPGTNRPPV (SEQ ID NO:1).

The reactivity to the TATTTTYAYPGTNRPPV peptide was further characterized using deletion and substitution peptide analogues. This analysis showed that the recognized region contained the sequence YAYPGTNRPPV (SEQ ID NO:2) (Table 6). Using alanine substitution analogues the Pro-Val sequence was found to the one most essential for the binding of human antibodies (Table 6).

Table 1 Complete amino acid sequences of the ORFs 1 and 2 of TTV (Genebank accession no AB008394) used for the synthesis of 80 overlapping peptides.

ORF1

MAYGWWRRRRRRWRRWRRRPWRRRWRTRRRRPARRRGRRRNVRRRRRGGRWRR
RYRRWKRKGRRRKKAKIIIRQWQPNYRRRCNIVGYIPVLICGENTVSRNYATHSDDT
NYPGPFGGGMTTDKFTLRILYDEYKRFMNYWTASNEDLDLCRYLGVNLYFFRHPDV
DFIIKINTMPPFLDTELTAPSIHPGMLALDKRARWIPSLKSRPGKKHYIKIRVGAPRMFT
DKWYPQTDLCDMVLLTVYATAADMQYPFGSPLTDSVVVNFQVLQSMYDKTISILPD
EKSQREILLNKIASYIPFYNTTQTIAQLKPFIDAGNVTSGATATTWASYINTTKFTTATT
TTYAYPGTNRPPVTMLTCNDSWYRGTVYNTQIQQLPIKAAKLYLEATKTLLGNTFTN
EDYTLEYHGGLYSSIWLSPGRSYFETTGAYTDIKYNPFTDRGEGNMLWIDWLSKKN
MNYDKVQSKCLISDLPLWAAAYGYVEFCAKSTGDQNIHMNARLLIRSPFTDPQLLVH
TDPTKGFVPYSLNFGNGKMPGGSSNVPIRMRAKWYPTLFHQQEVLEALAQSGPFAY
HSDIKKVSLGMKYRFKWIWGGNPVRQQVVRNPCKETHSSGNRVPRSLQIVDPKYNS
PELTFHTWDFRRGLFGPKAIQRMQQQPTTTDIFSAGRKRPRRDTEVYHSSQEGEQKES
LLFPPVKLLRRVPPWEDSQQEESGSQSSEEETQTVSQQLKQQLQQQRILGVKLRLLFN
QVQKIQQNQDINPTLLPRGGDLASLFQIAP

ORF2

MAEFSTPVRSGEATEGDLRVPRAGAEGEFTHRSQGAIRARDWPGYGQGSEKSMFIGR
HYRKKRALSLCAVRTTKKACKLLIVMWTPPRNDQHYLNWQWYSSILSSHAAMCGC
PDAVAHFNHLASVLRAPQNPPPPGPQRNLPLRRLPALPAAPEAPGDRAPWPMAGGAE
GEDGGAGGDADHGGAAGGPEDADLLDAVAAAE

TABLE 2. Analysis of human antibody reactivities in EIA to overlapping synthetic peptides corresponding to the complete open reading frame 1 (ORF1) of the TT virus. Values have been given as the optical density at 405 nm. OD values over 0.500 are considered positive and have been written in bold.

TTV ORF1 peptide	Samples negative for TTV DNA by PCR								Samples positive for TTV DNA by PCR							
	1	2	3	4	5	6	7	11	9	18	36	48	98	110	155	157
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	0.08	0.08	0.07	0.11	0.07	0.09	0.08	0.14	0.07	0.06	0.08	0.10	0.05	0.08	0.07	0.08
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
6	0.08	0.08	0.07	0.10	0.07	0.09	0.07	0.11	0.08	0.06	0.08	0.09	0.05	0.07	0.08	0.07
7	0.07	0.07	0.08	0.10	0.07	0.08	0.08	0.10	0.07	0.064	0.07	0.09	0.05	0.07	0.08	0.08
8	0.07	0.06	0.07	0.08	0.07	0.09	0.07	0.10	0.07	0.07	0.08	0.10	0.05	0.07	0.09	0.09
9	0.07	0.08	0.08	0.09	0.08	0.10	0.07	0.10	0.08	0.06	0.07	0.10	0.05	0.08	0.10	0.11
10	0.09	0.10	0.11	0.11	0.11	0.13	**1.09**	0.12	0.09	0.07	0.07	0.11	0.06	0.09	0.12	0.12
11	0.09	0.10	0.10	0.10	0.09	0.11	0.09	0.12	0.09	0.07	0.08	0.10	0.07	0.08	0.12	0.13
12	0.09	0.09	0.10	0.10	0.08	0.11	0.09	0.12	0.10	0.08	0.08	0.11	0.06	0.08	0.13	0.13
13	0.09	0.08	0.08	0.09	0.08	0.09	0.09	0.10	0.09	0.07	0.08	0.10	0.07	0.08	0.11	0.11
14	0.09	0.10	0.11	0.11	0.08	0.12	0.09	0.12	0.08	0.07	0.07	0.09	0.05	0.07	0.14	0.14
15	0.08	0.09	0.11	0.10	0.09	0.13	0.09	0.13	0.09	0.06	0.07	0.13	0.05	0.08	0.15	0.16
16	0.08	0.09	0.08	0.10	0.08	0.14	0.09	0.11	0.08	0.07	0.07	0.09	0.04	0.06	0.12	0.12
17	0.08	0.10	0.09	0.11	0.09	0.13	0.09	0.13	0.08	0.08	0.08	0.11	0.04	0.09	0.12	0.13
18	0.09	0.09	0.15	0.29	0.07	0.13	**0.62**	0.28	0.08	0.07	0.13	0.12	0.04	0.08	0.19	0.18
19	0.08	0.09	0.10	0.10	0.09	0.16	0.12	0.13	0.08	0.07	0.07	0.14	0.04	0.08	0.15	0.14
20	0.07	0.06	0.06	0.07	0.06	0.08	0.08	0.11	0.08	0.07	0.07	0.09	0.04	0.06	0.09	0.10
21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

22	0.07	0.07	0.07	0.08	0.08	0.09	0.09	0.11	0.08	0.07	0.08	0.10	0.04	0.07	0.11	0.11
23	0.09	0.09	0.11	0.11	0.08	0.16	0.09	0.12	0.08	0.07	0.07	0.10	0.05	0.07	0.14	0.14
24	0.07	0.09	0.12	0.12	0.08	0.12	0.08	0.11	0.08	0.07	0.07	0.10	0.05	0.10	0.13	0.15
25	0.07	0.09	0.08	0.08	0.06	0.11	0.07	0.12	0.07	0.06	0.06	0.06	0.06	0.07	0.11	0.12
26	0.07	0.09	0.07	0.08	0.08	0.10	0.07	0.10	0.07	0.07	0.06	0.05	0.07	0.08	0.09	0.11
27	0.07	0.08	0.07	0.08	0.06	0.11	0.07	0.08	0.06	0.06	0.06	0.06	0.08	0.06	0.09	0.10
28	0.07	0.08	0.08	0.08	0.07	0.50	0.07	0.10	0.07	0.06	0.06	0.06	0.07	0.06	0.09	0.10
29	0.42	0.36	0.45	**0.53**	0.22	**0.95**	0.29	0.14	0.21	0.11	0.09	0.10	0.09	0.25	0.59	0.62
30	0.07	0.07	0.06	0.07	0.06	0.09	0.07	0.09	0.07	0.07	0.07	0.07	0.08	0.08	0.09	0.12
31	0.05	0.09	0.09	0.08	0.06	0.09	0.07	0.08	0.06	0.06	0.06	0.06	0.06	0.06	0.09	0.09
32	0.06	0.08	0.06	0.25	0.06	0.09	0.06	0.07	0.06	0.05	0.06	0.05	0.07	0.06	0.09	0.09
33	0.07	0.08	0.06	0.07	0.06	0.08	0.06	0.07	0.06	0.05	0.06	0.05	0.07	0.06	0.08	0.09
34	0.07	0.07	0.05	0.06	0.06	0.06	0.06	0.07	0.06	0.09	0.06	0.15	0.06	0.06	0.08	0.08
35	0.18	0.17	0.27	**1.40**	0.25	**0.64**	**2.10**	**0.64**	0.10	0.10	**1.22**	**1.11**	0.05	0.47	**0.95**	**0.86**
36	0.07	0.07	0.06	0.07	0.06	0.11	0.07	0.08	0.09	0.06	0.06	0.07	0.07	0.07	0.08	0.08
37	0.10	0.11	0.11	0.12	0.09	0.14	0.12	0.14	0.09	0.08	0.08	0.20	0.10	0.08	0.14	0.14
38	0.07	0.08	0.08	0.09	0.08	0.11	0.09	0.11	0.08	0.07	0.07	0.07	0.09	0.08	0.19	0.11
39	0.08	0.09	0.07	0.09	0.07	0.37	0.09	0.10	0.08	0.09	0.09	0.08	0.09	0.09	0.11	0.13
40	0.09	0.16	0.24	0.12	0.11	0.16	0.09	0.12	0.09	0.09	0.08	0.08	0.10	0.09	0.15	0.16
41	0.08	0.12	0.10	0.11	0.10	0.20	0.09	0.15	0.09	0.07	0.09	0.08	0.11	0.09	0.15	0.16
42	0.08	0.11	0.09	0.10	0.17	0.23	0.11	0.19	0.27	0.12	0.10	0.09	0.16	0.42	0.35	**1.24**
43	0.09	0.21	0.11	0.11	0.10	0.41	0.10	0.14	0.08	0.08	0.08	0.08	0.12	0.11	0.20	0.25
44	0.09	0.11	0.11	0.12	**0.98**	0.15	0.10	0.14	0.09	0.09	0.08	0.08	0.12	0.13	0.17	**1.25**
45	0.08	0.06	0.06	0.12	0.10	0.17	0.10	0.14	0.09	0.08	0.06	0.09	0.11	0.09	0.16	0.17
46	0.08	0.08	0.07	0.09	0.08	0.22	0.10	0.12	0.08	0.07	0.07	0.07	0.09	0.08	0.19	0.26
47	0.07	0.09	0.08	0.09	0.09	0.13	0.09	0.12	0.07	0.07	0.07	0.07	0.10	0.09	0.15	0.19
48	0.08	0.11	0.10	0.12	0.10	0.16	0.11	0.13	0.08	0.09	0.08	0.08	0.12	0.09	0.15	0.15
49	0.09	0.11	0.10	0.11	0.10	0.15	0.15	0.15	0.08	0.08	0.07	0.08	0.10	0.11	0.26	0.25
50	0.07	0.09	0.08	0.09	0.08	**0.86**	0.29	0.18	0.08	0.07	0.07	0.07	0.10	0.20	**0.63**	0.46

51	0.06	0.09	0.08	0.10	0.12	0.24	0.13	0.13	0.07	0.07	0.08	0.08	0.11	0.09	0.24	0.78
52	0.10	0.13	0.11	0.11	0.10	0.20	0.11	0.13	0.10	0.09	0.08	0.08	0.14	0.11	0.21	0.38
53	0.12	0.12	0.09	0.10	0.08	0.16	0.09	0.11	0.09	0.10	0.08	0.07	0.09	0.10	0.16	0.15
54	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
55	0.10	0.10	0.07	0.08	0.07	0.14	0.09	0.08	0.35	0.08	0.07	0.07	0.08	0.09	0.14	0.12
56	0.10	0.16	0.11	0.11	0.09	0.18	0.09	0.11	0.10	0.09	0.09	0.09	0.10	0.10	0.18	0.15
57	0.14	0.12	0.10	0.12	0.09	0.17	0.11	0.12	0.11	0.08	0.09	0.09	0.10	0.12	0.19	0.43
58	0.07	0.06	0.06	0.07	0.06	0.07	0.07	0.08	0.07	0.06	0.08	0.08	0.04	0.06	0.08	0.09
59	0.06	0.05	0.05	0.06	0.06	0.06	0.06	0.08	0.06	0.05	0.05	0.06	0.04	0.05	0.07	0.08
60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
61	0.07	0.06	0.06	0.08	0.06	0.10	0.07	0.10	0.08	0.05	0.06	0.08	0.04	0.07	0.12	0.12
62	0.07	0.07	0.06	0.09	0.07	0.08	0.07	0.09	0.08	0.05	0.07	0.11	0.05	0.07	0.11	0.14
63	0.11	0.16	0.12	0.13	0.11	0.16	0.11	0.05	0.08	0.08	0.08	0.08	0.11	0.09	0.15	0.15
64	0.09	0.08	0.08	0.09	0.07	0.10	0.08	0.09	0.08	0.08	0.08	0.07	0.08	0.08	0.11	0.12
65	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
66	0.10	0.12	0.10	0.10	0.10	0.14	0.12	0.16	0.10	0.09	0.09	0.09	0.11	0.10	0.13	0.15
67	0.10	0.11	0.09	0.12	0.10	0.15	0.12	0.15	0.10	0.09	0.09	0.09	0.10	0.10	0.21	0.24
68	0.11	0.14	0.16	0.16	0.13	0.23	0.13	0.19	0.10	0.10	0.09	0.10	0.12	0.12	0.20	0.21
69	0.10	0.15	0.16	0.14	0.12	0.51	0.11	0.13	0.09	0.08	0.08	0.09	0.10	0.11	0.15	0.21
70	0.09	0.11	0.11	0.11	0.09	0.19	0.12	0.12	0.08	0.08	0.08	0.09	0.10	0.11	0.18	0.20
71	0.10	0.13	0.14	0.14	0.11	0.20	0.11	0.17	0.10	0.08	0.10	0.09	0.13	0.14	0.21	0.20
72	0.10	0.15	0.12	0.13	0.10	0.19	0.12	0.16	0.08	0.08	0.09	0.09	0.11	0.11	0.18	0.19
73	0.08	0.09	0.09	0.10	0.09	0.12	0.10	0.13	0.09	0.08	0.07	0.08	0.11	0.09	0.13	0.14
74	0.08	0.09	0.07	0.08	0.08	0.10	0.09	0.11	0.09	0.08	0.07	0.08	0.08	0.08	0.09	0.11
75	0.11	0.12	0.12	0.11	0.10	0.17	0.44	0.15	0.10	0.10	0.10	0.09	0.11	0.10	0.15	0.16
76	0.10	0.13	0.15	0.14	0.11	0.28	0.13	0.14	0.11	0.09	0.08	0.09	0.12	0.26	0.01	0.22
77	0.14	0.14	0.15	0.12	0.09	0.18	0.12	0.13	0.18	0.07	0.07	0.08	0.08	0.09	0.11	0.14

Table 3. Analysis of human antibody reactivities in EIA to overlapping synthetic peptides corresponding to the complete open reading frame 2 (ORF2) of the TT virus. Values have been given as the optical density (OD) at 405 nm. OD values over 0.500 are considered positive and have been written in bold.

TTV ORF2 peptide	Samples negative for TTV DNA by PCR								Samples positive for TTV DNA by PCR							
	1	2	3	4	5	6	7	11	9	18	36	48	98	110	155	157
1	0.09	0.10	0.13	0.11	0.10	0.16	0.09	0.12	0.08	0.08	0.09	0.08	0.08	0.08	0.14	0.14
2	0.10	0.11	0.11	0.10	0.08	0.12	0.09	0.14	0.08	0.08	0.08	0.08	0.08	0.09	0.14	0.13
3	0.08	0.10	0.11	0.10	0.08	0.29	0.28	0.14	0.10	0.08	0.13	0.08	0.11	0.10	0.14	0.04
4	0.09	0.09	0.08	0.08	0.07	0.09	0.07	0.09	0.08	0.07	0.07	0.07	0.07	0.07	0.10	0.10
5	0.08	0.08	0.08	0.08	0.07	0.10	0.08	0.10	0.07	0.07	0.07	0.07	0.07	0.08	0.12	0.13
6	0.07	0.10	0.08	0.10	0.07	0.10	0.07	0.10	0.08	0.28	0.09	0.08	0.10	0.09	0.09	0.11
7	0.11	0.10	0.11	0.14	0.91	0.13	0.10	0.11	0.08	0.09	0.10	0.11	0.10	0.09	0.10	0.13
8	0.09	0.10	0.11	0.13	0.10	0.12	0.10	0.14	0.09	0.08	0.10	0.11	0.10	0.10	0.11	0.12
9	0.08	0.09	0.09	0.09	0.09	0.10	0.09	0.11	0.09	0.08	0.08	0.09	0.09	0.09	0.13	0.14
10	0.09	0.11	0.10	0.09	0.08	0.11	0.08	0.10	0.09	0.09	0.07	0.08	0.08	0.08	0.11	0.13
11	0.10	0.10	0.11	0.08	0.09	0.09	0.09	0.11	0.10	0.12	0.09	0.07	0.09	0.09	0.14	0.14
12	0.12	0.09	0.09	0.08	0.09	0.09	0.08	0.07	0.13	0.09	0.08	0.10	0.14	0.12	0.16	0.14
13	0.07	0.09	0.09	0.08	0.08	0.10	0.09	0.11	0.07	0.08	0.07	0.08	0.09	0.08	0.12	0.12
14	0.08	0.07	0.08	0.07	0.07	0.08	0.08	0.10	0.07	0.07	0.07	0.07	0.07	0.07	0.10	0.10
15	0.08	0.08	0.08	0.07	0.07	0.09	0.07	0.10	0.08	0.07	0.07	0.07	0.08	0.08	0.10	0.12
16	0.08	0.09	0.09	0.07	0.07	0.09	0.08	0.09	0.08	0.08	0.08	0.07	0.08	0.08	0.12	0.11
17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
19	0.09	0.12	0.17	0.12	0.08	**0.51**	**0.57**	0.15	0.08	0.07	0.33	0.07	0.08	0.08	0.14	0.14
20	0.09	0.10	0.09	0.08	0.07	0.10	0.07	0.09	0.10	0.10	0.08	0.08	0.08	0.10	0.14	0.16

TABLE 4. Sequences of TTV peptides reactive with human serum samples.

ORF1

Peptide no.	Peptide sequence	
10	VLICGENTVSRNYATHS	SEQ ID NO:3
18	KINTMPPFLDTELTAPS	SEQ ID NO:4
29	PDEKSQREILLNKIASY	SEQ ID NO:5
35	TATTTTYAYPGTNRPPV	SEQ ID NO:1
42	GLYSSIWLSPGRSYFET	SEQ ID NO:6
44	YTDIKYNPFTDRGEGNM	SEQ ID NO:7
50	DQNIHMNARLLIRSPFT	SEQ ID NO:8
51	LIRSPFTDPQLLVHTDP	SEQ ID NO:9
69	QKESLLFPPVKLLRRVP	SEQ ID NO:10

ORF2

Peptide no.	Peptide sequence	
19	EDGGAGGDADHGGAAGGP	SEQ ID NO:11

TABLE 5. Analysis of the reactivities of serial dilutions of three human serum samples with to the TTV peptide TATTTTYAYPGTNRPPV (SEQ ID NO:1). Values are given as the OD and standard deviation (SD) at 405 nm.

Dilution of serum sample	**Human serum sample**					
	P 4	SD	P 6	SD	P 7	SD
1:100	1.285	0.072	0.687	0.082	1.782	0.054
1:200	0.758	0.056	0.375	0.003	1.23	0.02
1:400	0.411	0.021	0.19	0.007	0.79	0.018
1:800	0.234	0.008	0.104	0.003	0.45	0.002
1:1600	0.131	0.005	0.067	0.001	0.246	0.013
1:3200	0.076	0.003	0.049		0.131	0.006
1:6400	0.058	0.001	0.043		0.087	
1:12800	0.046		0.041		0.061	

TABLE 6. Analysis of the reactivities of three human serum samples with to the deletion and alanine substitution analogues of the TTV peptide TATTTTYAYPGTNRPPV (SEQ ID NO:1). Values are given as the OD at 405 nm. Positive reactivities, i.e. more than 50% of the reactivity of the original peptide, have been written in bold.

Deletion or substitution peptide analogue	Human serum sample		
	P4	P7	P36
TATTTTYAYPGTNRPPV	**0.839**	**1.845**	**0.825**
TATTTTYAYPGTNRPP	0.096	0.144	0.086
TATTTTYAYPGTNRP	0.100	0.099	0.078
TATTTTYAYPGTNR	0.092	0.103	0.078
TATTTTYAYPGTN	0.186	0.888	0.083
TATTTTYAYPGT	0.095	0.087	0.072
TATTTTYAYPG	0.095	0.085	0.074
TATTTTYAYP	0.095	0.096	0.082
TATTTTYAY	0.098	0.089	0.083
TATTTTYA	0.115	0.089	0.090
TATTTTY	0.142	0.105	0.076
TATTTT	0.108	0.093	0.082
TATTT	0.101	0.091	0.078
TATT	0.099	0.105	0.076
ATTTTYAYPGTNRPPV	**1.042**	**1.960**	**0.923**
TTTTYAYPGTNRPPV	**0.805**	**1.587**	**0.776**
TTTYAYPGTNRPPV	**0.697**	**1.488**	**0.810**
TTYAYPGTNRPPV	**0.748**	**1.659**	**0.722**
TYAYPGTNRPPV	**0.707**	**1.508**	**0.712**
YAYPGTNRPPV	**0.647**	**1.546**	**0.677**
AYPGTNRPPV	**0.662**	**1.488**	**0.669**
YPGTNRPPV	0.300	**1.091**	0.406
PGTNRPPV	0.166	0.430	0.123
GTNRPPV	0.300	0.887	0.210
TNRPPV	0.110	0.146	0.056
NRPPV	0.135	0.242	0.076
AATTTTYAYPGTNRPPV	**1.045**	**1.852**	**0.915**
TGTTTTYAYPGTNRPPV	**0.855**	**1.829**	**0.806**
TAATTTYAYPGTNRPPV	**0.897**	**1.675**	**0.764**
TATATTYAYPGTNRPPV	**0.971**	**1.722**	**0.824**
TATTATYAYPGTNRPPV	**1.076**	**1.867**	**0.955**
TATTTAYAYPGTNRPPV	**1.011**	**1.833**	**1.027**
TATTTTAAYPGTNRPPV	**0.898**	**1.619**	**0.901**
TATTTTYGYPGTNRPPV	**0.836**	**1.769**	**0.850**
TATTTTYAAPGTNRPPV	**0.899**	**1.697**	**0.903**
TATTTTYAYAGTNRPPV	**0.886**	**1.738**	**0.903**
TATTTTYAYPATNRPPV	**0.895**	**1.503**	**0.734**
TATTTTYAYPGANRPPV	**0.891**	**1.594**	**0.714**
TATTTTYAYPGTARPPV	**1.226**	**1.723**	**0.696**
TATTTTYAYPGTNAPPV	**0.761**	**1.558**	**0.708**
TATTTTYAYPGTNRAPV	**0.720**	**1.551**	**0.812**
TATTTTYAYPGTNRPAV	0.090	0.092	0.100
TATTTTYAYPGTNRPPA	0.108	0.105	0.095

References

1. Nishizawa T, Okamoto H, Konishi K, Yoshizawa H, Miyakawa Y, Mayumi M. A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. Biochem Biophys Res Commun 1997;241:92-7
2. Okamoto H, Akahane Y, Ukita M, Fukuda M, Tsuda F, Miyakawa Y, Mayumi M. Fecal excretion of a nonenveloped DNA virus (TTV) associated with posttransfusion non-A-G hepatitis. J Med Virol 1998;56:128-32
3. Cossart Y. TTV a common virus, but pathogenic? [comment]. Lancet 1998;352:164
4. Okamoto H, Kato N, Iizuka H, Tsuda F, Miyakawa Y, Mayumi M. Distinct genotypes of a nonenveloped DNA virus associated with posttransfusion non-A to G hepatitis (TT virus) in plasma and peripheral blood mononuclear cells [In Process Citation]. J Med Virol 1999;57:252-8
5. Naoumov NV, Petrova EP, Thomas MG, Williams R. Presence of a newly described human DNA virus (TTV) in patients with liver disease [see comments]. Lancet 1998;352:195-7
6. Viazov S, Ross RS, Varenholz C, Lange R, Holtmann M, Niel C, Roggendorf M. Lack of evidence for an association between TTV infection and severe liver disease [In Process Citation]. J Clin Virol 1998;11:183-7
7. Sällberg M, Ruden U, Magnius LO, Norrby E, Wahren B. Rapid "tea-bag" peptide synthesis using 9-fluorenylmethoxycarbonyl (Fmoc) protected amino acids applied for antigenic mapping of viral proteins. Immunology Letters 1991;30:59-68
8. Zhang ZX, Chen M, Hultgren C, Birkett A, Milich DR, Sällberg M. Immune responses to the hepatitis C virus NS4a are profoundly influenced by the combination of the viral genotype and the host major histocompatibility complex. J. Gen. Virol. 1997;78:2735-2746

CLAIMS

1. Peptide having the amino acid sequence

SEQ ID NO:1

Thr Ala Thr Thr Thr Thr Tyr Ala Tyr Pro Gly Thr Asn Arg Pro Pro Val

wherein one to six of the N-terminal amino acids Thr Ala Thr Thr Thr Thr may be omitted.

2. Peptide according to claim 1 having the amino acid sequence

SEQ ID NO:2

Tyr Ala Tyr Pro Gly Thr Asn Arg Pro Pro Val.

3. Peptide mixture comprising the peptide according to claim 1 and at least one of the peptides

SEQ ID NO:3

Val Leu Ile Cys Gly Glu Asn Thr Val Ser Arg Asn Tyr Ala Thr His Ser,

SEQ ID NO:4

Lys Ile Asn Thr Met Pro Pro Phe Leu Asp Thr Glu Leu Thr Ala Pro Ser,

SEQ ID NO:5

Pro Asp Glu Lys Ser Gln Arg Glu Ile Leu Leu Asn Lys Ile Ala Ser Tyr,

SEQ ID NO:6

Gly Leu Tyr Ser Ser Ile Trp Leu Ser Pro Gly Arg Ser Tyr Phe Glu Thr,

SEQ ID NO:7

Tyr Thr Asp Ile Lys Tyr Asn Pro Phe Thr Asp Arg Gly Glu Gly Asn Met,

SEQ ID NO:8

Asp Gln Asn Ile His Met Asn Ala Arg Leu Leu Ile Arg Ser Pro Phe Thr,

SEQ ID NO:9

Leu Ile Arg Ser Pro Phe Thr Asp Pro Gln Leu Leu Val His Thr Asp Pro,

SEQ ID NO:10

Gln Lys Glu Ser Leu Leu Phe Pro Pro Val Lys Leu Leu Arg Arg Val Pro, and

SEQ ID NO:11

Glu Asp Gly Gly Ala Gly Gly Asp Ala Asp His Gly Gly Ala Ala Gly Gly Pro.

4. Peptide according to claim 1 or 2, or a peptide mixture according to claim 3, wherein at least one peptide is coupled to a carrier and/or label.

5. Peptide according to claim 1 or 2, or a peptide mixture according to claim 3, wherein at least one peptide is immobilized on a solid phase.

6. Peptide or peptide mixture according to any one of the preceding claims for use in a medicament.

7. Monospecific antibody binding to the TT virus,

8. Monospecific antibody according to claim 7 binding to an amino acid sequence selected from the group consisting of the amino acid sequences SEQ ID NO:1 - 11.

9. Monospecific antibody according to claim 7 or 8 for use in a medicament.

10. Diagnostic kit comprising a peptide or peptide mixture according to any one of claims 1 - 5 as diagnostic antigen(s).

11. Diagnostic kit comprising one or more monospecific antibodies according to claim 7 or 8 as diagnostic antibodies.

12. Use of a peptide or peptide mixture according to any one of claims 1 - 4 for immunization of a non-human mammal to produce monospecific antibodies directed against TT virus.

SEQUENCE LISTING

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INTERNATIONAL SEARCH REPORT

Int. tional Application No
PCT/EP 00/03958

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/01 C07K16/08 G01N33/68 A61P31/20

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, STRAND

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	WO 99 58638 A (INNOGENETICS NV ;MAERTENS GEERT (BE); VREESE KAREN DE (BE)) 18 November 1999 (1999-11-18) claims 21-38 ---	1,6-12
A	WO 99 05282 A (NISHIZAWA TSUTOMU ;OKAMOTO HIROAKI (JP); TAMURA RYOJI (JP)) 4 February 1999 (1999-02-04) claims; examples -& EP 1 010 759 A 21 June 2000 (2000-06-21) ---	1,6-12

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 September 2000	13/09/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fuhr, C

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INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/EP 00/03958

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	DATABASE WPI Section Ch, Week 200036 Derwent Publications Ltd., London, GB; Class B04, AN 2000-415430 XP002146537 & JP 2000 135087 A (SRL KK), 16 May 2000 (2000-05-16) abstract -----	1,6-12

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte Jonal Application No
PCT/EP 00/03958

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9958638 A	18-11-1999	AU 4259299 A	29-11-1999
WO 9905282 A	04-02-1999	AU 8358698 A	16-02-1999
		EP 1010759 A	21-06-2000
		ZA 9809239 A	19-04-1999
JP 2000135087 A	16-05-2000	NONE	